or that these markers are not necessarily cartilage specific and could play similar or novel role in bone remodeling. Furthermore, the robust regulation of chondrogenic markers in bone and their good correlation to BMD & BMC suggest that they could be good predictors of disease and treatment outcomes.

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PHENOTYPIC CHARACTERIZATION OF OSTEOBLASTS FROM THE SCLEROTIC ZONE OF HUMAN OSTEOARTHRITIC SUBCHONDRAL BONE

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Purpose: There is consensus that osteoarthritis (OA) is characterized by subchondral bone thickening, accompanied by an increased osteoid volume and a low mineralization. Until now, phenotypical changes occurring in osteoblasts from the sclerotic subchondral bone remains unexplored. This work was designed to compare gene expression in osteoblasts coming from the sclerotic and non sclerotic zones of human OA subchondral bone.
Methods: Human osteoblasts were isolated from sclerotic or non sclerotic areas of OA subchondral bone. They were cultured for 12 days in monolayer in a differentiation medium composed of 2% Ultroser G as serum substitute, 2 mM proline, 50 microgram/ml ascorbic acid and 10^{-8} M 1,25 dihydroxycalciferol. At the end of this differentiation period, gene expression in sclerotic or non sclerotic osteoblasts was compared. Tissue non specific alkaline phosphatase (TNAP), osteocalcin (OC), transforming growth factor -beta1 (TGF-beta1), osteopontin (OPN), bone sialoprotein (BSP), vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-13, parathormone receptor (PTH-R), transglutaminase (TG)-2, factor XIIIA (FXIIIA), plasma cell membrane glycoprotein 1 (PC-1) and Ank mRNA levels were quantified using real time RT-PCR. Transglutaminase and nucleotide triphosphate pyrophosphohydrolase (NTPPPH) activities were also quantified by enzymatic assays.
Results: MMP-13 (21-fold; $p<0.001$), OPN (2.8-fold; $p<0.001$), TNAP (2-fold, $p<0.001$), OC (2-fold, $p<0.001$), TGF-beta1 (1.4-fold, $p<0.01$) and VEGF (1,5-fold; $p<0.001$) gene expression was significantly higher in sclerotic osteoblasts than in non sclerotic cells. In contrast, PTH-R (-37%, $p<0.001$), PC-1 (-22%, $p<0.01$), Ank (-24%, $p<0.01$) genes were depressed in sclerotic osteoblasts compared with non sclerotic cells. Finally, BSP, TG2 and FXIIIA mRNA levels were similar in sclerotic and non sclerotic osteoblasts. Transglutaminase activity was increased by 53% in sclerotic osteoblasts ($p<0.001$), while NTPPPH activity was decreased by 32% ($p<0.001$).
Conclusions: Osteoblasts from the sclerotic subchondral bone showed an altered phenotype characterized by the overexpression of genes limiting bone mineralization (OPN, PC-1, ...), on one hand, and genes promoting osteoid matrix accumulation (TGF-beta1, OC) on the other hand. These findings suggest that osteoblasts may contribute to subchondral bone sclerosis and as such constitute a potential target for future OA therapies.

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WNT SIGNALLING IN OSTEOARTHRITIC BONE

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Purpose: Wnts are secreted signalling molecules, traditionally associated with developmental processes. Various members of the Wnt signalling pathway have been implicated in osteogenic differentiation and high and low bone mass phenotypes. In osteoarthritis (OA) there is a massive proliferation of hypomineralized trabecular bone. Our hypothesis is that musculoskeletal cells revert to an earlier, developmental, phenotype and attempt to produce new matrix inappropriately. We have performed a pilot study to analyze the role of Wnt signalling in OA by profiling the expression of Wnt pathway genes in osteoblasts. Bone from patients with osteoporosis (OP), displaying a low bone mass phenotype, were used for comparison.
Methods: Femoral heads were obtained from consenting patients undergoing a total hip replacement for OA (N=5, aged 55-86) or a hemiarthroplasty following a fractured neck of femur for OP (N=5, aged 68-92). Primary osteoblasts were grown from bone chips. Total RNA was isolated from cells using Trizol (Invitrogen) followed by RNeasy purification (Qiagen) and prepared for application as biotinylated cRNA to a Wnt pathway Oligo GE Array (Super Array Biosciences) containing oligo-probes for 128 Wnt-related and housekeeping genes. Intensities were corrected for background and normalised using the inter-quartile median. Mean values of signal intensities (medians if not normally distributed) were found for each disease group, and compared using analysis of variance. The most highly expressed 30 genes (~25%) were examined in each group and the Log_2(OA/OP) (signal log ratio, SLR) calculated and deemed important if greater than 0.5 or less than -0.5.
Results: The Wnt signalling pathway was clearly active in osteoblasts in both dseases. Expression levels for the top 30 genes were significantly greater than those for the lowest expressed gene ($p<0.05$, pairwise comparison, ANOVA on ranks). WISP2, GSK3A, AES and DVL1 were among the most highly expressed genes in both diseases and 27 of the top 30 were common to both groups. High SLR values were found in 7 of these genes, though no differences in signal between the groups reached statistical significance at $p<0.05$. Secreted frizzled related proteins SFRP4 (SLR 0.84) ($p=0.056$), and SFRP3/FRZB (0.59) were higher in OA than OP. The Na/H transporter regulator SLC9A3R1/EBP50 (-0.85) and transducin-like enhancers of split TLE1 (-0.51) and TLE3 (-0.52) were more highly expressed in OP than OA. Of the Wnt proteins, Wnts 16, 1 and 5a were the most highly expressed but only WNT5A showed any differential expression with an SLR of 0.45 ($p=0.057$).
Conclusions: Wnt signalling is clearly active in elderly bone. Secreted frizzled-related proteins are extracellular inhibitors of Wnt signalling and, of particular interest, FRZB has been identified as a candidate in genetic linkage studies of OA. However, lower levels of TLEs in OA would indicate higher levels of gene transcription through the canonical signalling pathway. The balance between these processes needs further investigation. Dishevelled-dependent Wnt5a signalling can be transduced through the RHOA pathway, affecting the cytoskeleton and commitment to cell lineage, and via MAP kinases through the JNK pathway affecting gene transcription. Higher levels could be a factor underlying the cellular changes seen in OA.

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DIFFERENTIAL EXPRESSION OF GROWTH AND ANGIOGENESIS FACTORS IN PATIENTS WITH ASEPTIC OSTEONECROSIS OF THE FEMORAL HEAD AND PATIENTS WITH HIP OSTEOARTHRITIS

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Purpose: Aseptic osteonecrosis of the femoral head (ONFH) is a painful and progressive disorder of the hip, which often leads to collapse and disabling secondary osteoarthritis of the hip joint.